Tissue Factor and VEGF Expression in Prostate Carcinoma: A Tissue Microarray Study

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ABSTRACT

Tissue factor (TF) is the principal physiologic initiator of coagulation. It also plays an important role in tumor growth and metastasis possibly by contributing to angiogenesis. We evaluated the expression of TF in benign and malignant prostate tissue and correlated it with the expression of the pro-angiogenic protein, vascular endothelial growth factor (VEGF). We used a tissue microarray (TMA) constructed from 80 archival prostatectomy specimens. Core samples were collected from benign prostate tissue (BP) (n = 77), high-grade prostatic intraepithelial neoplasia (PIN) (n = 26), and carcinoma (PCa) (n = 93). TMA sections were stained with an immunopurified polyclonal TF antibody and a rabbit polyclonal VEGF. Two pathologists manually scored staining in epithelial cells using the German Immunoreactive Score. Positive staining for TF was seen predominantly in PCa with rare positive glands in BP and PIN. TF expression was significantly lower in BP versus PCa specimens (p < .001) and in PIN versus PCa specimens (p < .001). Positive staining for VEGF was seen in PCa, BP, and PIN. Rates of VEGF expression were also significantly lower in BP versus PCa specimens (p = .003) but not in PCa versus PIN (p = .430). The majority of PCa samples positive for TF were also positive for VEGF (p < .001). Our findings reinforce the link between angiogenesis and TF expression in PCa. We suggest further exploration of TF-mediated pathways leading to increased tumor aggressiveness in PCa, and the possible use of anti-TF agents in PCa.

INTRODUCTION

Prostate cancer (PCa) is the second most common cancer found in men, with a worldwide incidence of 25.3 per 100,000. The incidence has been increasing within the past decades mostly due to increased use of screening tests (1). The primary mechanisms in prostate carcinogenesis are slowly unfolding as researchers continue to delve into the pathways involved. One of the main areas of interest is the role of angiogenesis in prostate carcinogenesis.

Keywords: Tissue factor, Angiogenesis, Prostate cancer, VEGF

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Androgen, an important molecule in PCa growth, regulates angiogenesis via the modulation of an important proangiogenic cytokine, vascular endothelial growth factor (VEGF) (2). During androgen deprivation changes in effects on the vasculature, such as decreased blood flow and vascular regression, precede effects on the glandular compartment (3). In light of this, the persistence of angiogenesis in hormone-refractory PCa has led to a search for other cytokines that may modulate angiogenic proteins in these tumors and may be used as therapeutic targets.

Tissue factor (TF) is a 47-kDa transmembrane protein that is the principal physiologic initiator of coagulation (4). It also plays an important role in tumor growth and metastasis possibly by contributing to angiogenesis through the upregulation of VEGF and downregulation of the angiogenesis inhibitor thrombospondin, a mechanism independent of coagulation activation (5). In quiescent endothelium, TF is normally undetectable but is found in smooth muscle cells surrounding blood vessels. Conversely, TF has been detected in the neoplastic cells and endothelial cells of malignant tissue (6). TF has been implicated in cancer growth, metastasis, and hemostatic abnormalities found...
in most patients with advanced malignancies. TF expression is associated with increased angiogenesis in various solid neoplasms, such as hepatocellular, colorectal, and PCas (7–9), and also in hematologic malignancies (10). TF has also been found to be a useful prognostic factor in patients with metastatic PCa treated with androgen withdrawal therapy (11).

The relationship between TF and VEGF has been studied in other tumors but not in prostate carcinoma (9, 12). We have recently shown that TF expression in pancreatic cancer occurs early in malignant transformation and is associated with VEGF expression and possibly thromboembolism (12). In this study, we evaluated 80 prostatectomy specimens using a tissue microarray containing benign, preneoplastic, and malignant prostate tissue to study the differential expression of TF in prostate tissue in various stages of malignant transformation, and its relationship to angiogenesis in prostate carcinoma, as determined by VEGF expression.

**MATERIALS AND METHODS**

**Tissue microarray construction**

Prostate tissue microarray (PTMA) was constructed with approval from the URMC Research Subject Review Board as previously described (13, 14). In brief, the PTMA used 80 radical prostatectomy specimens from the archives of the Surgical Pathology Laboratory at the University of Rochester Medical Center – Strong Memorial Hospital. Areas containing benign prostatic tissue (BP) (n = 77), high-grade prostatic intraepithelial neoplasia (PIN) (n = 26), and PCa (n = 93) were marked for sampling. Multiple 0.6-mm core samples were retrieved from the selected region in each donor paraffin block and transferred to a TMA paraffin block. A TMA grid map was constructed to keep track of multiple core samples from the same prostatectomy specimen.

**Immunohistochemical staining**

Sections from the PTMA were deparaffinized, rehydrated through graded alcohols, and washed with TBS. Expression of TF and VEGF was determined using the streptavidin–biotin–peroxidase complex method with antigen retrieval, as reported previously (12).

Antibodies used for immunostaining included an immunopurified polyclonal IgG (anti-sTF; 0.5 mg/mL) against residues 1–218 of the extracellular domain of human TF (12), and rabbit polyclonal antibody to VEGF (1:50 dilution; Zymed Laboratories). Labeling for TF and VEGF was completed using DAKO Rabbit Envision Plus kit (DAKO, Carpenteria, CA). The sections were counterstained with a modified Mayer hematoxylin followed by 10 dips in 3% ammonia water. Lung parenchyma and an invasive ductal breast cancer with known VEGF positivity were used as positive controls for TF and VEGF, respectively.

**Immunohistochemical scoring**

Immunohistochemical scoring was performed using the German Immuno Reactive Score (15), which is calculated by combining the percentage of immunoreactive cells (quantity score) with an estimate of the staining intensity (staining intensity score) as follows: no staining is scored as 0, 1–10% of cells stained scored as 1, 11–50% as 2, 51–80% as 3, and 81–100% as 4. Staining intensity was rated on a scale of 0–3, with 0 = negative, 1 = weak, 2 = moderate, and 3 = strong. The raw data are converted to the immunoreactive score (IS) by multiplying the quantity and staining intensity scores. Cores with a score of IS of 0 or 1 are considered negative and those with IS ≥2 are considered positive. Each core was examined under a light microscope and separately scored. Cores that had <50% of designated tissue present were disregarded.

**Statistical analysis**

Data were first summarized in terms of their median, mean, standard deviation, and frequency. They were analyzed using mixed logistic regression models that included a random intercept as well as the following covariates: group, TF, and VEGF. Univariate analysis was conducted. Tissue expression of TF and VEGF were analyzed using Fisher’s exact test to determine statistically significant difference in expression between BP, PIN, and PCa.

**RESULTS**

**Immunohistochemical analysis of TF and VEGF expression**

TF expression was seen predominantly in the cytoplasm of neoplastic prostate glands with rare expression in benign glands and high-grade PIN [Figures 1(A) and (B)]. TF expression was identified in BP (9% positivity), PIN (8%), and PCa (47%). TF expression was significantly higher in specimens with PCa when compared with BP specimens (p < .001) and PIN specimens (p < .001) (Table 1). There was no significant difference in TF expression between BP and PIN (p = .473). Cytoplasmic expression of VEGF was likewise seen predominantly in the neoplastic prostate glands, benign glands, and high-grade

<table>
<thead>
<tr>
<th>Tissue factor</th>
<th>Positive (%)</th>
<th>Negative (%)</th>
<th>p Value</th>
</tr>
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<tbody>
<tr>
<td>BP</td>
<td>7 (9)</td>
<td>73 (91)</td>
<td>&lt;.001</td>
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<tr>
<td>PIN</td>
<td>2 (8)</td>
<td>23 (92)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>PCa</td>
<td>43 (47)</td>
<td>49 (53)</td>
<td></td>
</tr>
<tr>
<td>VEGF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BP</td>
<td>35 (45)</td>
<td>42 (55)</td>
<td>.003</td>
</tr>
<tr>
<td>PIN</td>
<td>13 (50)</td>
<td>13 (50)</td>
<td>.070</td>
</tr>
<tr>
<td>PCa</td>
<td>63 (68)</td>
<td>30 (32)</td>
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</tbody>
</table>

p value calculated vs. PCa.
Figure 1. Representative photomicrographs of TF staining. (A) Benign prostatic tissue with negative cytoplasmic staining. (B) Prostate carcinoma with positive cytoplasmic immunostaining. Representative photomicrographs of VEGF staining. (C) Benign prostatic tissue with negative cytoplasmic staining. (D) Prostate carcinoma with positive cytoplasmic immunostaining. Original magnification 20×, immunostain with hematoxylin counterstain.

PIN [Figures 1(C) and (D)]. There was significant difference in VEGF expression when comparing BP (35/77, 45%) versus PCa (63/93, 68%) specimens ($p = .003$) and with PCa versus PIN (13/26, 50%) specimens ($p < .070$) but not with BP versus PIN specimens ($p = .146$). Data is summarized in Table 1.

**Correlation of TF and VEGF expression**

VEGF expression was observed in 79% of PCa specimens with TF expression but only in 10% of PCa specimens without TF expression. Statistical analysis demonstrated a positive correlation between TF and VEGF using univariate logistic regression ($p < .001$).

**DISCUSSION**

The linkage between hemostasis and regulation of angiogenesis has been the focus of recent research (16). TF, the principal physiologic initiator of coagulation, may also be implicated in thrombosis during septic shock, atherosclerosis, and cancer (4). TF is also associated with tumor growth, angiogenesis, and invasion, especially in epithelial neoplasms. Recent studies suggest that TF-mediated signaling may play an important role in tumor angiogenesis as evidenced by the correlation between TF and VEGF expression in pancreatic and hepatocellular carcinoma (9, 12). TF influences the shift to an angiogenic phenotype in hematologic malignancies and may indirectly induce VEGF signaling through PAR1 signaling in stromal cells or by PAR1 activation of tumor cell (10). TF also regulates the expression of VEGF and the angiogenesis inhibitor thrombospondin (5). Yu et al. demonstrated that TF regulates VEGF expression in squamous cell carcinoma cells using C225, an antibody raised against epidermal growth factor receptor (EGFR) (17). This study established that TF downregulation by C225 was associated with a decrease in VEGF, indicating a relationship that may be triggered by activated oncogenes. One possible candidate oncogene, TF pathway inhibitor-2 (TFPI-2), is a structural homologue of TF pathway inhibitor and is frequently silenced by epigenetic alterations in several tumor cell lines, including gliomas, hepatocellular carcinoma, and breast carcinoma (18–21). Interestingly, these same tumor types also express TF, strengthening the
The authors declare no conflict of interest.

REFERENCES


